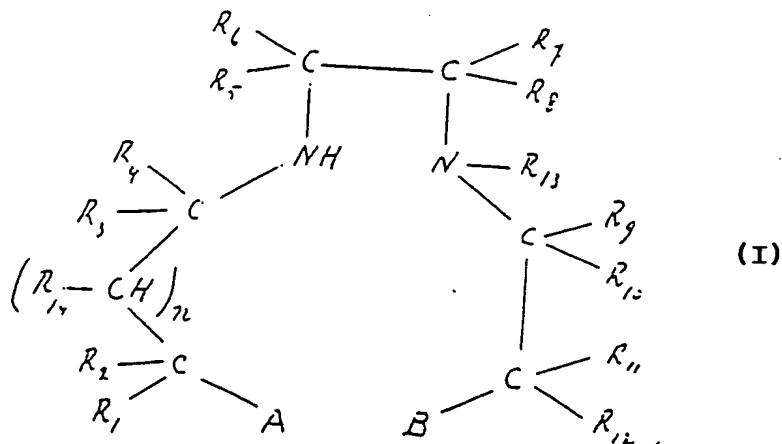




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A61K 43/00, 49/02	A1	(11) International Publication Number: WO 91/16076 (43) International Publication Date: 31 October 1991 (31.10.91)
(21) International Application Number: PCT/US91/02450 (22) International Filing Date: 10 April 1991 (10.04.91) (30) Priority data: 90200928.1 17 April 1990 (17.04.90) EP (34) <i>Countries for which the regional or international application was filed:</i> AT et al. (71) Applicant (for all designated States except US): MAL-LINCKRODT MEDICAL, INC. [US/US]; 675 McDonnell Blvd., P.O. Box 5840, St. Louis, MO 63134 (US). (71)(72) Applicant and Inventor: VERBRUGGEN, Alfons [BE/BE]; Herestraat 49, B-3000 Leuven (BE).		(74) Agents: HEY, David, A. et al.; Mallinckrodt Medical, Inc., 675 McDonnell Boulevard, P.O. Box 5840, St. Louis, MO 63134 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US. Published <i>With international search report.</i>

(54) Title: METHOD AND KIT FOR PREPARING TECHNETIUM-99M COMPLEXES**(57) Abstract**

The invention relates to a method of preparing a radiolabeled technetium complex by bringing together a pertechnetate solution in a complex-forming reaction with a compound of general formula (I) wherein R_1 - R_{12} independently represent H, C_1 - C_4 alkyl, a carboxy group, or carboxy (C_1 - C_4) alkyl; or wherein $R_1 + R_2$, $R_3 + R_4$, $R_5 + R_6$, $R_7 + R_8$, $R_9 + R_{10}$ and $R_{11} + R_{12}$ independently represent an oxo or imino function; and wherein: R_{13} is H, C_1 - C_4 alkyl or carboxy (C_1 - C_4) alkyl; R_{14} is H or NH_2 ; n is 0, 1 or 2; A represents NH_2 or SH; and B represents SH, or a group of the general formula $NH-(Z-CO-NH)_m-Z-COOH$; said reaction performed in the presence of Sn(II) as a reducing agent, in the absence of a transfer agent, in a substantially aqueous solvent system having a pH of at least 10, and at ambient temperature. The invention further relates to a kit for performing said method.

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Z is an optionally substituted C₁-C₄ alkylene, cycloalkylene or alkylidene group, and

m is an integer from 0 to 20;

with the provisos:

- (i) that at least one substituent selected from the group consisting of R_1 - R_{13} and B is or comprises a carboxy group; and
- (ii) that R_3+R_4 or R_7+R_8 represents an oxo function or both R_3+R_4 and R_7+R_8 or both R_5+R_6 and R_7+R_8 represent oxo functions, if A is an amino group and B is a group of the formula $NH\leftarrow Z-CO-NH\rightleftarrows_m Z-COOH$.

If Z is a substituted alkylene, cycloalkylene or alkylidene group, said group is substituted with one or more substituents selected from phenyl; phenyl substituted with one or more substituents selected from hydroxy, halogen and substituted phenoxy; hydroxy; mercapto; thio; methylthio; carboxy; imino; amino; amido; imidazolyl; and indolyl.

Such a complex-forming reaction as defined above, starting, however, from a compound of formula I wherein optionally mercapto groups are protected, is described in European patent application publ. no. 173424. According to this patent publication, Tc-99m mercaptoacetyltriglycine is prepared by heating a mixture of benzoyl mercaptoacetyltriglycine, an exchange ligand, a Sn(II) salt as a reducing agent and a Tc-99m pertechnetate solution in a boiling water bath for five minutes. According to European patent application, publ. no. 250013, related compounds like Tc-99m mercaptoacetylglucylserylglucine are prepared in a corresponding manner, viz. by heating the S-benzoyl mercapto compound together with a Sn(II) salt, Tc-99m pertechnetate and a buffer solution (pH 5.0) in a boiling water bath for 10 minutes. According to this latter European patent publication such technetium-99m complexes can also be prepared starting from the corresponding ethyl ester, viz. by heating said

ester in a boiling water bath in the presence of sodium dithionite as a reducing agent in the presence of a base, apparently to saponify the ester to the corresponding acid. As a matter of fact, however, Sn(II) salts are preferred to dithionite as reducing agent in the labelling reaction with Tc-99m pertechnetate, because dithionite is not sufficiently stable under the normal storage conditions and therefore less attractive for pharmaceutical purposes.

In various respects the reaction conditions for the preparation of the radiolabelled technetium complex, as described in the above patent publications, are unsatisfactory. Usually the labelling reaction is carried out by the user himself, applying Tc-99m pertechnetate from a molybdenum-technetium generator that is at his disposal. It stands to reason that such operations to be carried out with radioactive material under aseptic conditions, which operations should often be performed in a clinic or clinical laboratory, should be as simple as possible to avoid any risks as to contamination of the pharmaceutical composition to be prepared and as to accidental radioactive contamination of the environment. Use of a boiling water bath for performing the above reaction is therefore a disadvantage. It would be highly favorable to be able to perform said complex-forming reaction at room temperature. Such favorable reaction conditions, however, should not be to the detriment of the reaction time. Preferably the labelling reaction should result in the desired product in a high yield only after 5 to 10 minutes at room temperature. Another disadvantage is the application of a so-called transfer agent, also defined as transfer ligand or exchange ligand, which is generally used to perform the desired complex-forming reaction. Although the labelling yield may sometimes be improved by using such a transfer agent, said agent will contaminate the final pharmaceutical composition and generally has an adverse

influence on the reaction rate. Moreover, it is a disadvantage that in the complex-forming reaction described in the above European patent applications a compound is used as a starting material wherein the mercapto group is protected, e.g. by a benzoyl group. As a matter of fact, this protective group should be removed and thus may contaminate the final product.

It is the object of the present invention to provide a method of preparing a radiolabelled technetium complex, in which the above disadvantages do not occur.

This object can be achieved by a method as defined in the opening paragraph, which method is characterized according to the present invention, in that said complex-forming reaction is performed in the presence of Sn(II) as a reducing agent, in the absence of a transfer agent, in an at least substantially aqueous solvent system having a pH of at least 10, and at ambient temperature.

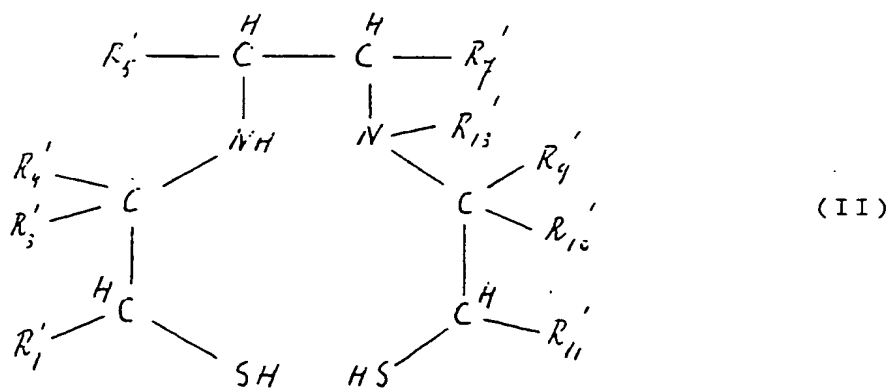
It has been found that under the above favorable reaction conditions of the present invention indeed in a reaction time of 5 to 10 minutes the complex-forming reaction is complete, yielding the desired radiolabelled product in a high yield and purity. Examples of suitable Sn(II) salts which can be used as reducing agents in the above reaction are stannous chloride and stannous fluoride.

A basic environment for the radioactive labelling of certain amine ligands with technetium-99m has been investigated by Volkert et al. (J. Appl. Radiat. Isot. 33, 1982, 891-896) and by Troutner et al. (J. Nucl. Med. 21, 1980, 443-448). In the reaction described by Volkert et al., however, a ligand transfer is included, resulting in a reaction time of 30 minutes to accomplish the desired ligand exchange. The used exchange ligands are tartrate, DTPA and citrate. Troutner has used cyclam as a complexing ligand, both with and without tartrate as an exchange ligand. Reaction

temperature and reaction time are not mentioned in this publication. It should be emphasized, however, that cyclam as a ligand for technetium-99m is not well comparable with the complexing compound of the general formula I defined above.

Cyclam is a tetra-azacyclo-tetradecane, so a macrocyclic compound having four secondary amino functions. Therefore cyclam is to be considered as a strong base which undergoes easily deprotonation at a high pH. The compound of formula I to be used for complexing Tc-99m in the method of the invention, on the contrary, comprises, in addition to at least one carboxy group, at least one mercapto function and/or amide function for chelating the radioactive metal ion, which functions do not show basic characteristics. Hence the complexing characteristics of cyclam differ fundamentally from those of the compound of general formula I as defined above, as also appears from the structure of the final technetium-99m complex formed.

More in particular the present invention relates to a method as defined hereinbefore, wherein for the complex-forming reaction preferably is used a compound of the general formula



wherein R₃', R₄', R₉' and R₁₀' each independently represents a hydrogen atom, a methyl group, a carboxy group or a carboxymethyl group;

or wherein $R_3' + R_4'$ and $R_9' + R_{10}'$ each independently represent an oxo function;

and wherein furthermore:

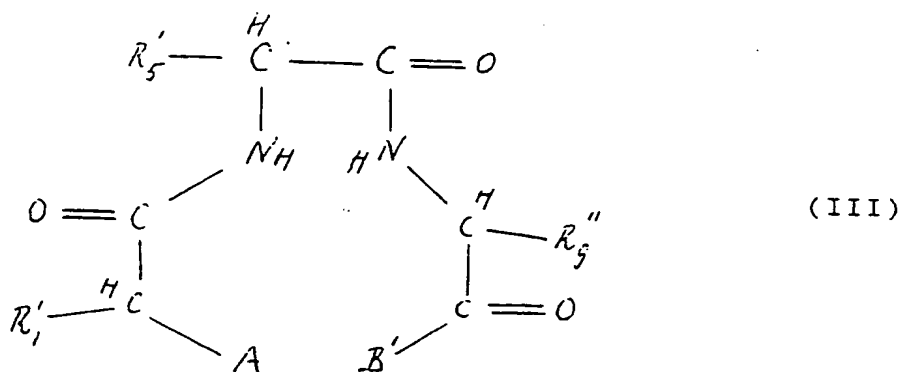
R_1' , R_5' , R_7' and R_{11}' each independently represents a hydrogen atom, a methyl group, a carboxy group or a carboxymethyl group; and

R_{13}' is a hydrogen atom, a methyl group or a carboxymethyl group;

with the proviso, that at least one substituent selected from the group consisting of R_1' , R_3' , R_4' , R_5' , R_7' , R_9' , R_{10}' , R_{11}' and R_{13}' is or comprises a carboxy group.

Examples of the latter compounds are N_2S_2 compounds, such as carboxy group-comprising diaminedithiols or dimercaptodiamides, for example 2,3-bis(mercaptoacetyl-amino)propanoic acid and 1,2-ethylenedicysteine.

The method of the invention is also particularly suitable for complexing Tc-99m with a compound of the general formula



wherein R_1' , R_5' and R_9'' each independently represents a hydrogen atom, a methyl group, a carboxy group or a carboxymethyl group;

A has the above meaning; and

B' represents a group of the general formula $NH-Z'-COOH$, wherein Z' is a C_1 - C_4 alkylene or alkylidene group.

Suitable examples of the last-mentioned preferred compounds are disclosed in the European patent applications mentioned before, viz. publ. nos. 173424 and 250013, like mercapto-acetyltriglycine (MAG3), tetraglycine and mercaptoacetyl-glycylalanylglycine (MAGAG). It is indeed a real surprise that such compounds, which comprise even three amide functions for chelating Tc-99m, so readily complex this metal-radionuclide ion at room temperature under the conditions defined above.

It has been found that the last-mentioned preferred method can be performed excellently at a pH of at least approximately 12.

As mentioned before, it is often more convenient to place the material to be labelled at the user's disposal, viz. in the form of a so-called kit, so that the user himself can prepare the desired radiolabelled technetium complex. For this purpose, the various non-radioactive ingredients are then offered to the user in the form of a "cold" kit. As asserted above, the operations to be carried out by the user should be as simple as possible, in order to enable him to prepare from said kit the Tc-99m labelled compound by using the facilities that are at his disposal.

Because the method of the present invention can be performed in a so simple and easy manner, viz. even without heating the reaction mixture, said preparation method can be carried out very well by the user himself. Therefore the invention also relates to a kit for performing said method, comprising (i) in an optionally dry condition a compound as defined hereinbefore, to which, if desired, an inert pharmaceutically acceptable carrier and/or a formulating agent and /or one or more auxiliary substances is/are added, (ii) a Sn(II) salt (as a reducing agent), (iii) a basic substance, said ingredients (i), (ii) and/or (iii) optionally being combined, (iv) a neutralizing agent in the form of an

acid or buffering substance, and (v) instructions for use with a prescription for reacting ingredients (i), (ii) and (iii) of the kit with technetium-99m in the form of a pertechnetate solution as described above, followed by a neutralization to a pH suitable for intravenous or subcutaneous administration by adding said agent defined sub (iv). Suitable neutralizing agents defined sub (iv) are mineral acids like hydrochloric acid and phosphoric acid, or buffer solutions such as phosphate buffer, acetate buffer or citrate buffer.

The ingredient of the kit mentioned sub (i) may be supplied as a solution, for example in the form of a physiological saline solution, or in some buffer solution, but may also be present in a dry condition, for example in a lyophilized condition. When used as a component for an injection liquid, this ingredient may be in a sterile condition, or, alternatively, the injection liquid may be sterilized before administration. In case the above ingredient is in a dry condition, the user may use a sterile physiological saline solution as a solvent therefor. If desired, the above-mentioned ingredient may be stabilized in the usual way with suitable stabilizers like ascorbic acid, gentisic acid or salts of these acids, or may be provided with other auxiliaries like fillers.

The invention will now be described in more detail with reference to the ensuing specific examples.

EXAMPLE I

Preparation of Tc-99m MAG3 starting from benzoylmercaptoacetyltriglycine

A. Preparation of labelling kits

To 25 mg benzoylmercaptoacetyltriglycine is added 25 ml 0.1 molar phosphate buffer pH 10.5 and the mixture is heated for 10 min in a water-bath at 100°C. After cooling 2.5 mg $\text{SnCl}_2 \cdot \text{H}_2\text{O}$ dissolved in 0.5 ml EtOH is added and the pH is

adjusted to pH 12 with NaOH 1N. The solution is diluted to 25 ml with water and dispensed in 1 ml-aliquots in reaction vials. The labelling kits are lyophilized, stoppered under vacuum and then stored at 4-8°C.

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B. Preparation of Tc-99m MAG3

To a labelling kit, obtained as described under (A), is added 1 ml to 6 ml generator eluate, containing 10 mCi to 100 mCi Tc-99m in the form of pertechnetate. The contents are mixed and the vial is incubated for 5 min at room temperature.

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C. Analysis of the labelling reaction mixture by reverse phase HPLC and TLC

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Twenty five μ l of the reaction mixture, obtained as described under Example IB, is applied at the top of a Hypersil[®] 5 μ m ODS column (250 mm x 4.6 mm) that is eluted with gradient mixtures of 0.0125 M phosphate buffer pH 5.85 and ethanol (0 min to 15 min: 0% to 20% ethanol, 15 min to 20 min: 20% to 50% ethanol, 20 min to 30 min: 50% to 90% ethanol).

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Radioactivity in the eluate is monitored by a 2 inch NaI(Tl)scintillation detector, coupled to an amplifier, single channel analyzer and Ramona[®] integrating system. A typical retention time of Tc-99m MAG3 is about 13 min, depending on the configuration of the HPLC-system. This retention time is identical to that of Tc-99m MAG3 obtained by exchange labelling in the presence of tartrate. The preparation is analyzed for colloidal technetium-99m by TLC on ITLC-SG, eluted with acetonitrile-water (1:1). Typical labelling yields using the described procedure are 95-98%. Labelling is unsuccessful if the pH during labelling is inferior to pH 10.

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EXAMPLE II

Preparation of Tc-99m MAG3 starting from benzylmercap-
toacetyltriglycine

5 A. Conversion of benzylmercaptoacetyltriglycine to mercaptoa-
 cetyltriglycine

10 0.500 g benzylmercaptoacetyltriglycine is dissolved in 250
 ml of liquid ammonia in a two-neck 500 ml-flask equipped
 with a cooling condenser (-75°C) and magnetic stirrer. To
 the mixture is added 125 mg Na and the mixture is stirred
 for 15 min. The cooling condenser is then removed and the
 ammonia is allowed to evaporate under a stream of argon. The
 residue is stored under nitrogen.

15 B. Labelling of MAG3 with technetium-99m

20 To a solution of 1 mg MAG3, prepared as described under
 Example IIA, in 1 ml NaOH 0.1 N is added 100 µg SnCl₂·2H₂O
 dissolved in 25 µl ethanol and 2 ml generator eluate,
 containing 10 mCi - 100 mCi Tc-99m in the form of pertech-
 netate. The mixture is incubated at room temperature for 10
 min. Analysis of Tc-99m MAG3 is performed by the HPLC and
 TLC methods described under Example IC. Typical labelling
 yields are 88%-91% Tc-99m MAG3.

25 EXAMPLE III

Labelling of 2,3-bis(mercaptoacetyl-amino)propanoic acid with
 Tc-99m

30 A. Conversion of 2,3-bis-(benzylmercaptoacetyl-amino)-
 propanoic acid to 2,3-bis(mercaptoacetyl-amino)propanoate
 (CO₂-DADS)

 The same procedure is used as described under Example IIA,
 but twice the amount of sodium is used.

B. Labelling

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To a solution of 1 mg CO₂-DADS in 1 ml NaOH 0.1 N is added 100 µg SnCl₂ dissolved in 25 µl ethanol and 2 ml generator eluate, containing 10 mCi - 100 mCi Tc-99m in the form of pertechnetate. The mixture is incubated at room temperature for 10 min and then neutralized by the addition of 0.5 M phosphate buffer pH 5. Analysis is performed by the HPLC and TLC methods described under Example IC. Typical labelling yields are 85-90% Tc-99m CO₂-DADS.

10 Example IV

Labelling of 1,2-ethylenedicysteine with technetium-99m

A. Labelling

To a solution of 1 mg 1,2-ethylenedicysteine in 1 ml 0.05 molar phosphate buffer pH 12 is added a solution of 100 µg SnCl₂·2H₂O in 25 µl HCl 0.05 M and 1 ml to 4 ml generator eluate (10 mCi to 100mCi Tc-99m in the form of pertechnetate). The mixture is incubated for 5 min at room temperature.

20 B. Analysis

25 µl of the reaction mixture is applied at the top of a Hypersil 5 µm ODS column (250 mm x 4.6 mm), that is eluted with gradient mixtures of 0.025 M phosphate buffer pH 2.5 and ethanol (0 min to 6 min : 0% to 9% ethanol; 6 min to 25 min : 9% ethanol; 25 min to 30 min : 9% to 0% ethanol). The radioactivity in the eluate is monitored by a 2 inch NaI(Tl) scintillation detector coupled to an amplifier, single channel analyzer and Ramona[®] integrator. A typical retention time of Tc-99m-ethylenedicysteine is 12 min. The reaction mixture is also analyzed for the presence of colloidal technetium-99m by TLC on ITLC-SG, eluted with acetonitrile-water (1:1). Typical labelling yields in the described conditions are 98-99%. The labelling yield decreases significantly as the pH during labelling is

inferior to pH 11, viz. to approx. 69 % at pH 9 and to approx. 47 % at pH 7.

Example V

5 A. Labelling of a tetrapeptide, viz. tetraglycine, with technetium-99m

To a solution of 1 mg tetraglycine in 1 ml 0.1 N NaOH is added a solution of 100 μ g Sn Cl₂.2H₂O in 25 μ l HCl 0.05 N and 1 ml to 4 ml generator eluate containing 10 mCi to 100 mCi Tc-99m in the form of pertechnetate. The mixture is incubated for 5 min at room temperature.

B. Analysis of the reaction mixture by RP-HPLC and TLC

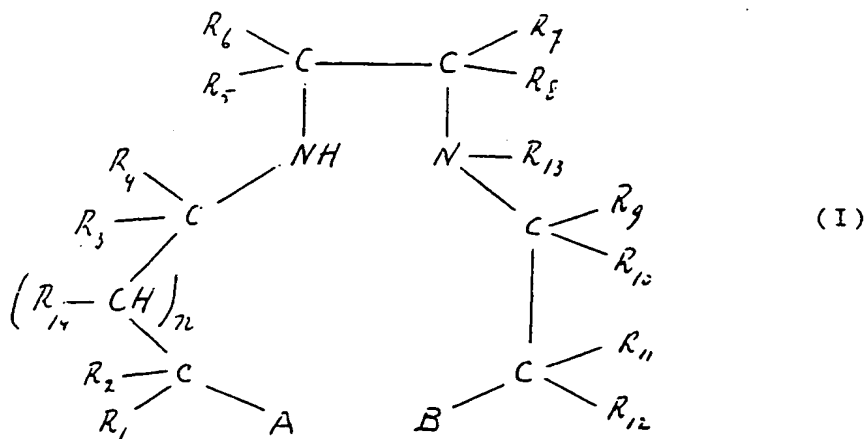
15 25 μ l of the reaction mixture is applied at the top of a Hypersil 5 μ m ODS column (250 mm x 4.6 mm) that is eluted with gradient mixtures of 0.05 M phosphate buffer pH 5.85 and ethanol as described under Example IC.

A typical retention time of the Tc-99m tetraglycine complex in the described conditions is 12 min, but it can vary depending on the HPLC-configuration and column characteristics. The reaction product is also analyzed for the presence of colloidal technetium-99m (TcO₂) by TLC on ITLC-SG, eluted with saline or acetonitrile-water (1:1). Typical yields are 94-98%.

25 By the same procedure other tetrapeptides are labelled with Tc-99m, e.g. alanyltriglycine, glycylalanyldiglycine and tetra-alanine. Labelling yields are in each case over 90%.

Claims

1. A method of preparing a radiolabelled technetium complex by bringing Tc-99m in the form of a pertechnetate solution in a complex-forming reaction with a compound of the general formula



wherein R_1 - R_{12} each independently represents a hydrogen atom, a C_1 - C_4 alkyl group, a carboxy group, or a carboxy(C_1 - C_4)alkyl group;

or wherein

R_1+R_2 , R_3+R_4 , R_5+R_6 , R_7+R_8 , R_9+R_{10} and $R_{11}+R_{12}$ each independently represent an oxo or imino function;

and wherein furthermore:

R_{13} is a hydrogen atom, a C_1 - C_4 alkyl group or a carboxy(C_1 - C_4)alkyl group;

R_{14} is a hydrogen atom or an amino group;

n is 0, 1 or 2;

A represents an amino group or a mercapto group; and

B represents a mercapto group, or a group of the general formula $NH-(Z-CO-NH)_mZ-COOH$,

wherein:

Z is an optionally substituted C_1 - C_4 alkylene, cycloalkylene or alkylidene group, and

m is an integer from 0 to 20;

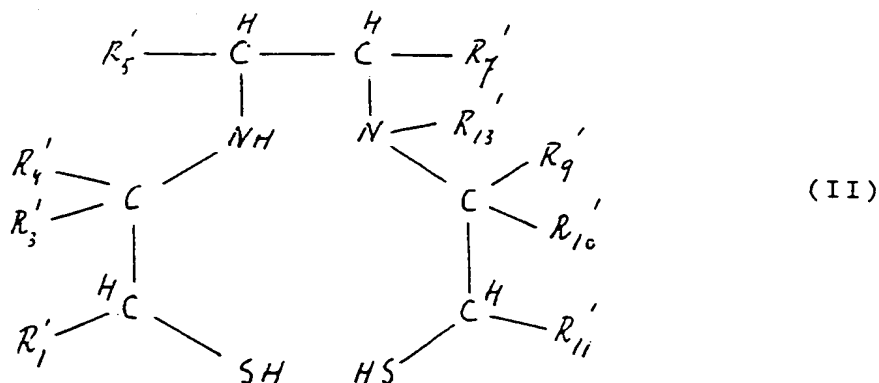
with the provisos:

(i) that at least one substituent selected from the group consisting of R_1 - R_{13} and B is or comprises a carboxy group; and

(ii) that R_3+R_4 or R_7+R_8 represents an oxo function or both R_3+R_4 and R_7+R_8 or both R_5+R_6 and R_7+R_8 represent oxo functions, if A is an amino group and B is a group of the formula $NH-(Z-CO-NH)_mZ-COOH$;

characterized in that said complex-forming reaction is performed in the presence of $Sn(II)$ as a reducing agent, in the absence of a transfer agent, in an at least substantially aqueous solvent system having a pH of at least 10, and at ambient temperature.

2. A method as claimed in claim 1, by bringing Tc-99m in the form of a pertechnetate solution in a complex-forming reaction with a compound of the general formula



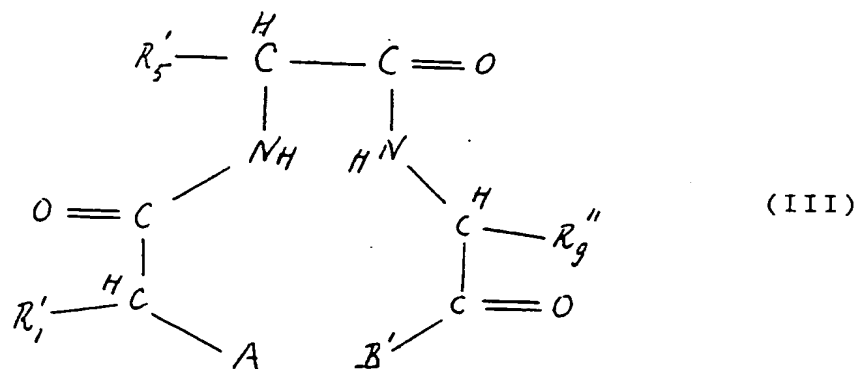
wherein R_3' , R_4' , R_9' and R_{10}' each independently represents a hydrogen atom, a methyl group, a carboxy group or a carboxymethyl group;
or wherein $R_3'+R_4'$ and $R_9'+R_{10}'$ each independently represent an oxo function;
and wherein furthermore:

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R_1' , R_5' , R_7' and R_{11}' each independently represents a hydrogen atom, a methyl group, a carboxy group or a carboxymethyl group; and R_{13}' is a hydrogen atom, a methyl group or a carboxymethyl group;

with the proviso, that at least one substituent selected from the group consisting of R_1' , R_3' , R_4' , R_5' , R_7' , R_9' , R_{10}' , R_{11}' and R_{13}' is or comprises a carboxy group; characterized in that said complex-forming reaction is performed in the presence of Sn(II) as a reducing agent, in the absence of a transfer agent, and in an at least substantially aqueous solvent system having a pH of at least 10.

3. A method as claimed in claim 1, by bringing Tc-99m in the form of a pertechnetate solution in a complex-forming reaction with a compound of the general formula



wherein R_1' , R_5' and R_9'' each independently represents a hydrogen atom, a methyl group, a carboxy group or a carboxymethyl group;

A has the meaning given in claim 1; and

B' represents a group of the general formula $NH-Z'-COOH$, wherein Z' is a C_1 - C_4 alkylene or alkylidene group;

characterized in that said complex-forming reaction is performed in the presence of Sn(II) as a reducing agent, in the absence of a transfer agent, and in an at least substantially aqueous solvent system having a pH of at least 10.

4. A method as claimed in claim 3, characterized in that the complex-forming reaction is performed at a pH of at least approximately 12.

5. A kit for performing a method as claimed in claim 1, comprising (i) in an optionally dry condition a compound of the general formula I, as presented in claim 1, wherein the symbols have the meanings given in claim 1, to which compound, if desired, an inert pharmaceutically acceptable carrier and/or a formulating agent and /or one or more auxiliary substances is/are added, (ii) a Sn(II) salt (as a reducing agent), (iii) a basic substance, said ingredients (i), (ii) and/or (iii) optionally being combined, (iv) a neutralizing agent in the form of an acid or buffering substance, and (v) instructions for use with a prescription for reacting ingredients (i), (ii) and (iii) of the kit with technetium-99m in the form of a pertechnetate solution as described in claim 1, followed by a neutralization to a pH suitable for intravenous or subcutaneous administration by adding said agent defined sub (iv).

6. A kit as claimed in claim 5, comprising as said ingredient (i) in an optionally dry condition a compound of the general formula II or III, as presented in claim 2 or 3, respectively, wherein the symbols have the meanings given in these claims, to which compound, if desired, an inert pharmaceutically acceptable carrier and/or a formulating agent and/or one or more auxiliary substances is/are added.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/02450

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC(5): A61K 43/00, 49/02																							
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched ⁷</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 20%; border: 1px solid black; padding: 5px;">Classification System</th> <th style="border: 1px solid black; padding: 5px;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; padding: 5px; vertical-align: top;"> U.S. IPC(5) </td> <td style="border: 1px solid black; padding: 5px; vertical-align: top;"> 424/1.1, 534/14 A61K 43/00, 49/02 (1978 to present) </td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸</div>			Classification System	Classification Symbols	U.S. IPC(5)	424/1.1, 534/14 A61K 43/00, 49/02 (1978 to present)																	
Classification System	Classification Symbols																						
U.S. IPC(5)	424/1.1, 534/14 A61K 43/00, 49/02 (1978 to present)																						
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; border: 1px solid black; padding: 5px;">Category [*]</th> <th style="width: 70%; border: 1px solid black; padding: 5px;">Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²</th> <th style="width: 20%; border: 1px solid black; padding: 5px;">Relevant to Claim No. ¹³</th> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="border: 1px solid black; padding: 5px; vertical-align: top;"> US, A, 4,571,430 (BYRNE ET AL) 18 FEBRUARY 1986 *column 6, line 54 bridging column 7, line 15* *Example 5, column 9*. </td> <td style="border: 1px solid black; text-align: center; vertical-align: top; padding: 5px;">1-4</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="border: 1px solid black; padding: 5px; vertical-align: top;"> US, A, 4,638,051 (BURNS ET AL) 20 JANUARY 1987. *column 6, lines 30-60* </td> <td style="border: 1px solid black;"></td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="border: 1px solid black; padding: 5px; vertical-align: top;"> US, A, 4,789,736 (CANNING ET AL) 06 DECEMBER 1988 *Examples 21, column 13* </td> <td style="border: 1px solid black;"></td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="border: 1px solid black; padding: 5px; vertical-align: top;"> US, A, 4,818,813 (NOWOTNIK ET AL) 04 APRIL 1989 *column 3, lines 4-17* *column 6, line 1-28* </td> <td style="border: 1px solid black;"></td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="border: 1px solid black; padding: 5px; vertical-align: top;"> US, A, 4,849,511 (VERBRUGGEN) 18 JULY 1989 *Example II, column 6* </td> <td style="border: 1px solid black;"></td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top; padding: 5px;">Y,P</td> <td style="border: 1px solid black; padding: 5px; vertical-align: top;"> US, A, 4,925,650 (NOSCO ET AL) 15 MAY 1990 *column 5 lines 25-56* *Examples II, column 7* </td> <td style="border: 1px solid black; text-align: center; vertical-align: top; padding: 5px;">1-6</td> </tr> </table>			Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	Y	US, A, 4,571,430 (BYRNE ET AL) 18 FEBRUARY 1986 *column 6, line 54 bridging column 7, line 15* *Example 5, column 9*.	1-4	A	US, A, 4,638,051 (BURNS ET AL) 20 JANUARY 1987. *column 6, lines 30-60*		A	US, A, 4,789,736 (CANNING ET AL) 06 DECEMBER 1988 *Examples 21, column 13*		A	US, A, 4,818,813 (NOWOTNIK ET AL) 04 APRIL 1989 *column 3, lines 4-17* *column 6, line 1-28*		A	US, A, 4,849,511 (VERBRUGGEN) 18 JULY 1989 *Example II, column 6*		Y,P	US, A, 4,925,650 (NOSCO ET AL) 15 MAY 1990 *column 5 lines 25-56* *Examples II, column 7*	1-6
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>[*] Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"d" document member of the same patent family</p> </div> </div>																							
IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border: 1px solid black; padding: 5px; vertical-align: top;"> Date of the Actual Completion of the International Search <div style="text-align: center; font-size: 1.2em;">02 JULY 1991</div> </td> <td style="width: 50%; border: 1px solid black; padding: 5px; vertical-align: top;"> Date of Mailing of this International Search Report <div style="text-align: center; font-size: 1.2em;">22 JUL 1991</div> </td> </tr> <tr> <td style="border: 1px solid black; padding: 5px; vertical-align: top;"> International Searching Authority <div style="text-align: center; font-size: 1.2em;">ISA/US</div> </td> <td style="border: 1px solid black; padding: 5px; vertical-align: top;"> Signature of Authorized Officer <div style="text-align: center;"> NGUYEN NGOC-HO INTERNATIONAL DIVISION <i>for</i> JOHN M. COVERT <i>Nguyen Ngoc Ho</i> </div> </td> </tr> </table>			Date of the Actual Completion of the International Search <div style="text-align: center; font-size: 1.2em;">02 JULY 1991</div>	Date of Mailing of this International Search Report <div style="text-align: center; font-size: 1.2em;">22 JUL 1991</div>	International Searching Authority <div style="text-align: center; font-size: 1.2em;">ISA/US</div>	Signature of Authorized Officer <div style="text-align: center;"> NGUYEN NGOC-HO INTERNATIONAL DIVISION <i>for</i> JOHN M. COVERT <i>Nguyen Ngoc Ho</i> </div>																	
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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A,P

US, A, 4,988,496 (SRINIVASAN ET AL) 29 JANUARY 1991
 Example II, column 34, lines 53-60

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter ¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹³, specifically:

3. ☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.